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Prospective Study of Mother-to-Infant Transmission of Hepatitis C Virus (HCV) Infection

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Seventy-five women with anti-hepatitis C virus (HCV) antibody were enrolled prospectively during pregnancy or at delivery for study of mother-to-child transmission of HCV. Twenty-three women were coinfected with the human immunodeficiency virus (HIV).

Seventy babies were monitored for at least 6 months. HCV infection was diagnosed in six infants (8.6%), four of whom were born to anti-HIV–positive mothers. HCV RNA was first detected between 2 and 6 months, and the genotypes of infected babies matched those of their mothers (type 1: n = 4; type 3: n = 2). Identical master sequences of the hypervariable region (HVR1) were detected in a mother–infant pair. In three babies coinfected with HCV and HIV, anti-HCV disappeared between 2 and 7 months, being persistently negative in two cases monitored for 11 and 26 months. Transmitting mothers did not differ significantly from those who did not transmit the infection with anti-HIV, HCV genotypes, and viral load at delivery, but had lower rate of reactivity to C100 by the recombinant immunoblot assay (RIBA) ($P < .01$).

This prospective study confirms transmission of HCV from anti-HIV–negative mothers (4.4% in this series). Absence of anti-C100 antibodies at delivery is apparently related to increased risk of vertical transmission. Seronegative HCV infection can be observed in children coinfected with HIV. ***J. Med. Virol. 54:12–19, 1998.***

KEY WORDS: paediatric HCV infection; HCV/HIV coinfection; HCV RNA titer; HCV genotypes; recombinant immunoblot assay; HCV hypervariable region

INTRODUCTION

The hepatitis C virus (HCV) is a major cause of liver disease. Like hepatitis B virus (HBV) and the human immunodeficiency virus (HIV), HCV is transmitted by percutaneous inoculation, but a high percentage of infected subjects have no identified exposure source. Maternal transmission of HCV has been reported previously [Aizaki et al., 1996; Lin et al., 1994; Manzini et al., 1995; Ni et al., 1994; Novati et al., 1992; Ohto et al., 1994; Thaler et al., 1991; Weiner et al., 1993; Zanetti et al., 1995], and increased rates of transmission were found in infants of mothers coinfected with HIV and HCV [Thaler et al., 1991; Novati et al., 1992; Zanetti et al., 1995]. However, conflicting results were reported on the frequency of mother-to-infant HCV transmission, possibly due to the different populations studied and to the tests used to monitor infants of HCV-infected mothers.

To determine the incidence of vertical HCV infection and to evaluate the maternal factors related to the risk of transmission, a prospective study was carried out of babies born to anti-HCV–positive women, with and without evidence of HIV infection.

PATIENTS AND METHODS

Study Population

Seventy-five women with anti-HCV (mean age 29.8 ± 5.3 years) were enrolled between January 1994 and January 1996, either during pregnancy or at delivery, for the study of mother-to-infant HCV transmission. All women were informed of the aim of the study. Anti-HCV was detected before pregnancy in 31 women and during pregnancy in 43 women. In one case acute HCV infection was documented during the 2nd month of

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pregnancy. Liver biopsy results, available in six cases, showed mild chronic active hepatitis in four patients and chronic persistent hepatitis in two.

The study protocol included determination of anti-HCV by enzyme-linked immunosorbent assay (ELISA), recombinant immunoblot assay (RIBA), and serum HCV RNA at the time of delivery. Mother-to-infant transmission of HCV infection was monitored by anti-HCV by ELISA and by serum HCV RNA at least twice during follow-up. Samples positive with anti-HCV were also tested by RIBA.

Serological Tests

Anti-HCV antibodies were assayed in duplicate by third-generation ELISA and RIBA (Ortho Diagnostic Systems, Raritan, NJ). Anti-HIV antibodies were determined by HIV1/HIV2 ELISA (Ortho Diagnostic Systems). HBsAg was detected by commercially available kits (Abbott Laboratories, North Chicago, IL).

Amplification of HCV RNA by Polymerase Chain Reaction (PCR)

Serum samples were stored at −80°C until used. HCV RNA was determined by PCR using the Amplicor HCV kit (Roche Diagnostic Systems, Branchburg, NJ) following the manufacturer's instructions. The determination of viral genotype was performed by differential hybridization of the 5′ untranslated region (UTR) [Ravaggi et al., 1996]. The PCR protocol used for amplification of the E1/E2 region has been detailed previously [Ravaggi et al., 1994].

Quantification of HCV RNA

The determination of HCV RNA copy number in serum samples was carried out by a competitive PCR-differential hybridization assay [Ravaggi et al., 1995]. A standard RNA containing two point mutations compared to the 5′ UTR sequence was added in known amount to each sample. After reverse transcription and nested PCR, each sample was subjected to differential hybridization on microtiter plates, using probes specific for wild-type and competitor sequences. The ratio between the optical density values obtained on each probe, plotted on a standard curve, allowed the determination of HCV RNA titer.

Cloning and Nucleotide Sequence Analysis

The PCR products were cloned in the Bluescript plasmid vector (Stratagene, La Jolla, CA). Nucleotide sequence analysis was carried out on denatured double-stranded DNA using the dideoxy-chain termination method. Both strands were entirely sequenced for each clone using the Sequenase version 2.0 kit (United States Biochemical Corp., Cleveland, OH). To analyze the degree of sequence heterogeneity of the hypervariable region 1 (HVR1), seven individual clones were derived from each sample.

Sequence data were analyzed by a computer programme (PC/GENE, Intelligenetics, Geel, Belgium).

Statistical Analysis

The characteristics of patients were compared by parametric or non-parametric tests as appropriate, using the EPI.INFO 5.0 statistical package (Centers for Disease Control, Atlanta, GA). A P value of .05 or less was considered statistically significant.

RESULTS

HBsAg was detected in three (4%) and anti-HIV in 23 (30.7%) of the 75 anti-HCV–positive women enrolled in this study. The stage of HIV infection was determined by using the 1993 Revised Centers for Disease Control (CDC) system. Infection status was A1 in ten cases, A2 in eight cases, and A3 in 5. Risk factors for infection included intravenous drug abuse (n = 35, 47%), history of blood transfusion(s) (n = 16, 21%), and anti-HCV–positive partner (n = 4, 5%). No known risk factor for infection was reported by 20 women (27%). The prevalence of risk factors was significantly different between anti-HIV–positive and –negative mothers ($P < .01$) (Table I).

The RIBA was reactive (presence of at least two bands) in 71 women (94.7%) and indeterminate in four. Reactivity against C22 was present in all cases, whereas anti-C33 antibody was detected in 92%, anti-C100 antibody in 69%, and anti-NS5 antibody in 41%. The percentage of reactivity against single antigens was not influenced by the anti-HIV status, with the exception of anti-NS5 antibody that was less frequent in anti-HIV–positive mothers ($P < .05$).

Serum HCV RNA was detected in 63 women (84%), 43 out of 52 anti-HIV negative women (83%) and 20 out of 23 anti-HIV positive women (87%). The viral RNA was detected in 88.7% of RIBA-reactive mothers and in none of RIBA indeterminate ones ($P < .01$). Anti-C33 was statistically associated with the presence of HCV RNA, being detected in 61 out of 63 viremic mothers (97%) and in eight of 12 mothers negative for HCV RNA (66.6%) ($P < .01$).

The viral genotype was determined in all women positive for HCV RNA, detecting genotype 1 in 37 cases (59%), genotype 2 in 13 (20.5%), genotype 3 in 12 (19%) and genotype 4 in 1 (1.5%). The distribution of viral genotypes was significantly different between anti-HIV positive and negative mothers ($p < 0.05$). HCV RNA titer was determined at delivery in 42 women (median 7.28, range 5–8.55 log copies/ml). The viral load was not related to the anti-HIV status (Table I).

Of 79 infants born during the study period (including 3 sibling pairs and 1 set of twins), 70 (88.6%) were monitored for at least six months (mean follow-up time 15.4 ± 6.9 months, range 6–32). All babies were anti-HCV positive at birth, with RIBA profile similar to the mother. At 5–8 months of age, anti-HCV was detected by ELISA in 52 out of 66 samples available (78.8%). RIBA was performed in 51 ELISA-positive samples, resulting reactive in 23 (45%), indeterminate in 27 (53%) and negative in 1 (2%). Five out of 41 infants (12.2%) were positive by ELISA at 10–12 months. Of

TABLE I. Characteristics of Anti-HIV–negative and –Positive Mothers*

	Anti-HIV−	Anti-HIV+	Total	*P*
n (%)	52 (69.3)	23 (30.7)	75 (100)	
Age (means ± SD)	30.8 ± 5.6	28 ± 4.4	29.8 ± 5.3	NS
Risk factors, n (%)				<.01
IVDU	15 (29)	20 (87)	35 (47)	
T	16 (31)	0	16 (21)	
S	1 (2)	3 (13)	4 (5)	
NN	20 (38)	0	20 (27)	
Anti-HCV RIBA, n (%)				
Reactive	48 (92.3)	23 (100)	71 (94.7)	NS
Indeterminate	4 (7.7)	0	4 (5.3)	NS
C100	35 (67.3)	17 (73.9)	52 (69)	NS
C22	52 (100)	23 (100)	75 (100)	NS
C33	46 (88.5)	23 (100)	69 (92)	NS
NS5	26 (50)	5 (21.7)	31 (41)	<.05
HCV RNA n (%)	43 (83)	20 (87)	63 (84)	NS
Genotypes, n (%)				<.05
1	23 (51.2)	15 (75)	37 (59)	
2	13 (30.2)	0	13 (20.5)	
3	7 (16.3)	5 (25)	12 (19)	
4	1 (23.3)	0	1 (1.5)	
Titer (log copies/ml)				NS
No. tested	23	19	42	
Median	7.3	7.2	7.28	
Range	5–8.55	5.1–8	5–8.55	

*SD, standard deviation; NS, not significant; IVDU, intravenous drug abuse; T, transfusion; S, seropositive partner; NN, unknown.

these, 2 were reactive and 3 indeterminate by RIBA. Only 1 out of 36 infants tested after the age of 13 months was still reactive by ELISA and RIBA (Fig. 1). Timing of anti-HCV disappearance was not affected by the anti-HIV status of the mother (data not shown).

The two infants reactive by ELISA and RIBA after the age of 10 months were both positive for HCV RNA. On the whole, HCV RNA was detected during follow-up in 6 out of 70 infants monitored for at least 6 months (8.6%), 4 of which born to anti-HIV positive mothers. Three of these babies were coinfected with HCV and HIV. The rate of HCV transmission was 4.4% from anti-HIV negative mothers and 18.2% from anti-HIV positive mothers.

To analyze the possible risk factors for vertical transmission, the mothers of the 6 infants who acquired HCV were compared with the 61 mothers whose 64 infants had been followed for at least 6 months without evidence of infection (Table II). Maternal risk factors for infection, anti-HIV status, presence of HCV RNA, viral genotype, and RNA titer were not statistically related to the occurrence of vertical HCV transmission. One out of six mothers who transmitted HCV infection and 45 out of 61 mothers who did not had anti-C100 antibody ($P < .01$). The percentage of reactivity to other antigens in the RIBA assay did not differ significantly between the two groups, although all mothers who transmitted HCV infection were negative for anti-NS5 antibody. None of the mothers of HCV-infected babies was positive for HBsAg or had a clinical history of HCV infection before pregnancy. The occurrence of HCV transmission by HIV-infected mothers did not appear to be related to maternal HIV stage (data not shown). All six HCV-infected babies were born by normal transvaginal delivery, whereas 19 mothers of uninfected babies had a caesarean section (P = NS). Only one mother that transmitted HCV infection breast-fed her baby, as did 26 out of 61 non-transmitting mothers (P = NS).

TABLE II. Characteristics of Mothers Who Transmitted HCV Infection*

	Transmitting	Non-transmitting	*P*
n (%)	6 (9)	61 (91)	
Age (means ± SD)	29.7 ± 3.7	30.2 ± 5.4	NS
Risk factors, n (%)			NS
IVDU	5 (83.3)	28 (45.9)	
T	1 (16.7)	14 (23)	
S	0	3 (4.9)	
NN	0	16 (26.2)	
Anti-HIV, n (%)	4 (66.7)	18 (29.5)	NS
Anti-HCV RIBA, n (%)			
C100	1 (16.7)	45 (73.8)	<.01
C22	6 (100)	61 (100)	NS
C33	6 (100)	5 (91.8)	NS
NS5	0	27 (45.1)	NS
HCV RNA, n (%)	6 (100%)	52 (85.2)	NS
Genotypes, n (%)			NS
1	4 (66.7)	31 (59.6)	
2	0	12 (23.1)	
3	2 (33.3)	9 (17.3)	
Titer (log copies/ml)			NS
No. tested	5	36	
Median	7.2	7.35	
Range	6.8–7.3	5–8.1	

*SD, standard deviation; NS, not significant; IVDU, intravenous drug abuse; T, transfusion; S, seropositive partner; NN, unknown.

The serological and clinical presentation of HCV infection in the six vertically infected babies (five females and one male) is shown in Figure 2. The four babies tested at birth were positive by ELISA and RIBA and negative for HCV RNA. The viral RNA was first de-

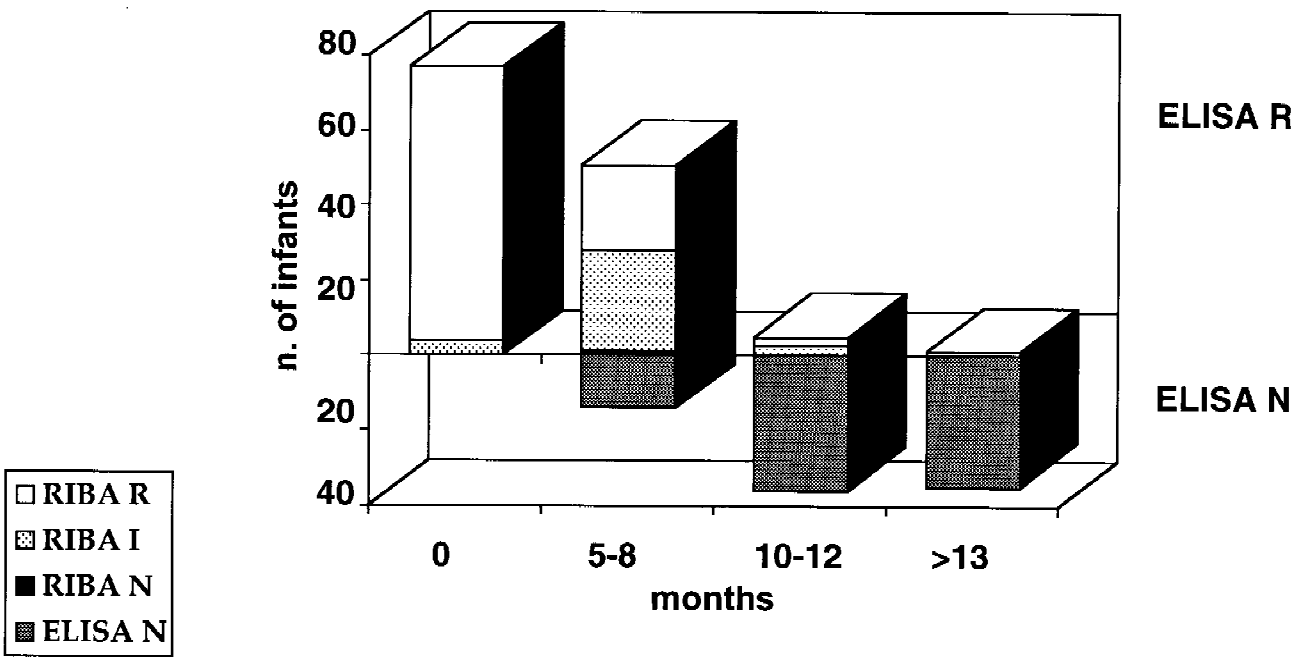

Fig. 1. Time-course of HCV serology in infants of anti-HCV–positive mothers. R, reactive; I, indeterminate; N, negative.

tected between 2 and 6 months of age and was persistently positive in all cases except one (A.C.). Two infants co-infected with HIV (C.J. and A.C.) resulted indeterminate on RIBA at 1–2 months and persistently anti-HCV negative thereafter, until 26 and 11 months of follow-up, respectively. In the remaining case with concurrent HIV infection (S.J.) the RIBA pattern was persistently indeterminate with reactivity to C22 from 1 to 6 months. Anti-HCV antibodies disappeared at 7 months, and seroconversion to anti-C33 was detected at 8 months. Antibodies to HCV were present in all available samples from children without HIV infection (G.S., M.J., and Z.F.). One infant (M.J.) was transiently indeterminate on RIBA at 3 and 4 months.

The profile of alanine aminotransferase (ALT) activity in infants with vertical HCV infection was variable, but abnormal values (>50 IU/L) were detected in all cases on at least one occasion. None of the HCV-infected babies developed HBsAg, or IgM antibodies to hepatitis A virus, cytomegalovirus (CMV), or Epstein-Barr virus (EBV). Five did not become icteric. ALT levels persistently twofold above normal limits were observed from 4 months onward in one infant coinfected with HIV (A.C.), who died at 11 months with signs of cholestatic hepatitis and of liver failure. Liver biopsy, carried out at the age of 8 months, had shown the presence of acute hepatitis. Immunohistochemical staining for CMV and EBV antigens was negative.

HCV sequences of the E1–E2 junction, including the hypervariable region 1, were derived from a mother–infant pair seronegative for HIV and infected with HCV genotype 3 (Fig. 3). HCV RNA sequences were derived from serum samples collected from the mother at delivery and from the infant (M.J.) at 3 months. The same master sequence was isolated from both mother and infant, consistent with the occurrence of vertical transmission. Five out of the seven clones isolated from the mother had identical sequences, one had a silent mutation in the E1 gene, and one had two mutations leading to amino acid changes in the HVR1. Only two of the clones derived from the infant were identical with those isolated from the mother's serum. The five remaining sequences had mutations in the HVR1 (n = 4, three of which were silent) or in the E2 gene (n = 1, leading to an amino acid substitution).

DISCUSSION

The occurrence of vertical HCV infection was documented unequivocally by previous reports [Aizaki et al., 1996; Lin et al., 1994; Manzini et al., 1995; Ni et al., 1994; Novati et al., 1992; Ohto et al., 1994; Thaler et al., 1991; Weiner et al., 1993; Zanetti et al., 1995], but the frequency of transmission from anti-HIV-negative mothers, the maternal factors associated with transmission, and the possible occurrence of seronegative infection in infants are still debated.

This prospective study showed vertical HCV infection in 8.6% of children born to anti-HCV–positive mothers, the rate of transmission increasing to 10.3% for babies whose mothers were positive for serum HCV RNA at delivery. These results are consistent with previous prospective studies carried out in Italy [Zanetti et al., 1995] and Japan [Ohto et al., 1994].

Several reports showed an increased frequency of HCV infection in children born to mothers with concurrent HIV infection, whereas the risk of vertical HCV transmission from anti-HIV–negative mothers was considered extremely low [Zanetti et al., 1995; Roudot-

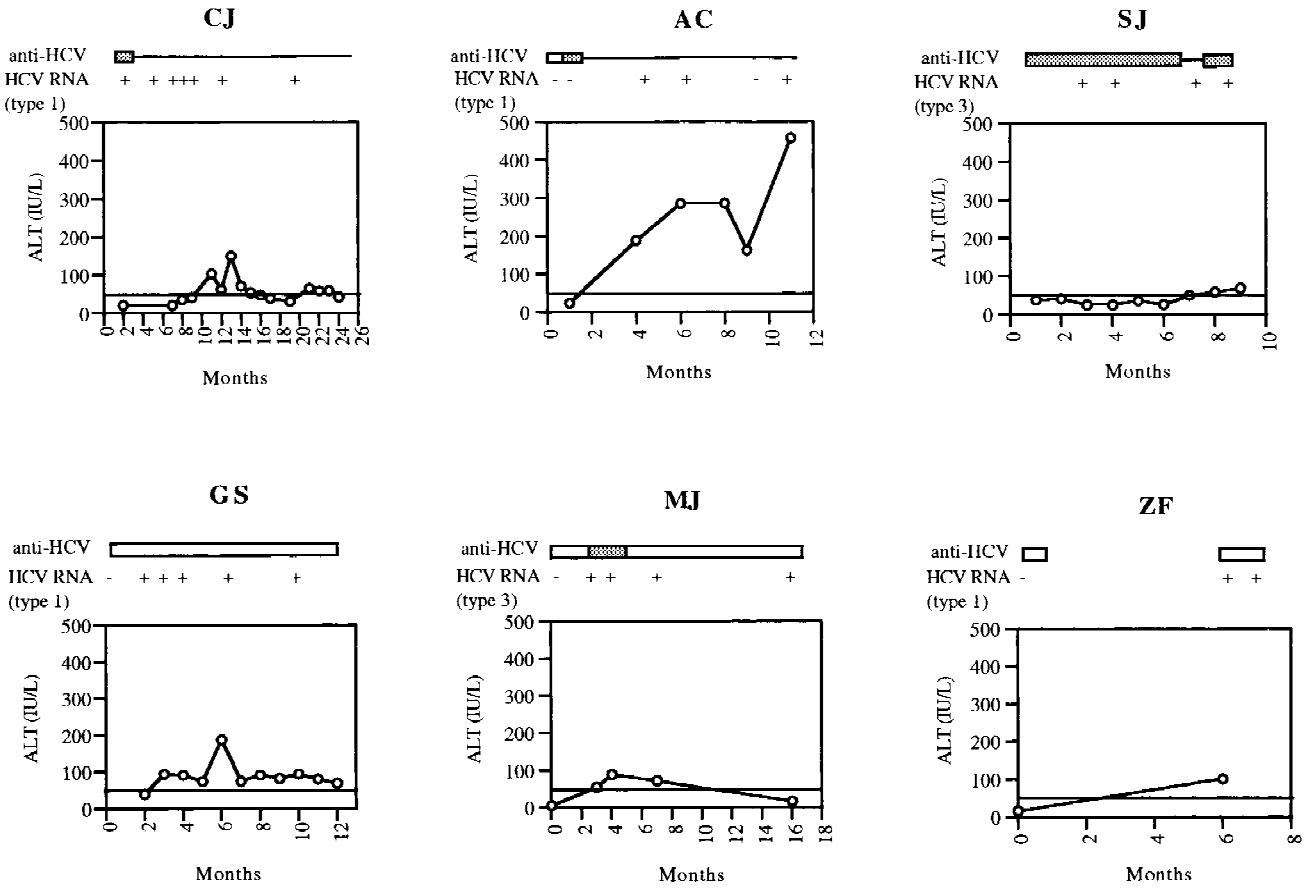

Fig. 2. Time-course of anti-HCV (top), HCV RNA, and ALT activities in six infants perinatally infected with HCV. C.J., A.C., and S.J. were coinfected with HIV. Open bars: anti-HCV RIBA reactive; dotted bars: anti-HCV RIBA indeterminate; line: anti-HCV (ELISA and RIBA) negative.

Thoraval et al., 1993]. These observations have been related to the presence of higher HCV RNA titer in subjects coinfected with HIV [Zanetti et al., 1995]. Although our results showed increased frequency of infection from anti-HIV–positive mothers (18.2%), a statistical correlation was not found between presence of anti-HIV and HCV transmission. The HCV genotype distribution was correlated with HIV status, as already reported [Ravaggi et al., 1996], but significantly higher HCV RNA titer in HIV-infected mothers was not detected. This might be due to the characteristics of the study population, including subjects with early stage of HIV infection.

Other factors may play a role in HCV mother-to-child transmission. Independent of anti-HIV status, enhanced risk of vertical HCV infection was related to high maternal HCV RNA titer [Ohto et al., 1994; Lin et al., 1994], exposure to parenterally or sexually transmitted diseases [Thaler et al., 1991], and previous diagnosis of chronic hepatitis [Ohto et al., 1994]. The HCV RNA titer and genotype, risk factors, and clinical history of HCV infection did not differ between transmitting or non-transmitting mothers included in this study. Surprisingly, a low rate of reactivity against C100 on RIBA was statistically correlated with increased rate of transmission. It has been reported that the presence of neutralizing antibodies to HIV in seropositive mothers is associated with the lack of mother-to-child HIV transmission [Scarlatti et al., 1993]. HCV may induce virus-neutralizing antibodies, as suggested by the detection of circulating immune complexes in chronic hepatitis C [Hijikata et al., 1993] and their relationship with reduced infectivity both in vivo and in vitro [Hijikata et al., 1993; Shimizu et al., 1993, 1994]. Since neutralizing antibodies to enveloped viruses are almost always directed against the envelope proteins, it is unlikely that anti-C100, which recognizes the nonstructural protein NS4, may have virus-neutralizing properties. However, our results suggest that anti-C100 might represent a surrogate marker for a presently uncharacterized antibody, which might be associated with lower infectivity titer independent of genomic RNA levels.

Consistent with previous results it was found that all newborns had passively acquired maternal anti-HCV antibodies, which disappeared by 12 months. However, between 5 and 8 months the rate of reactivity by ELISA and RIBA had decreased markedly. The persistence of anti-HCV antibodies after 12 months is generally considered to be a marker of infection, and the existence of

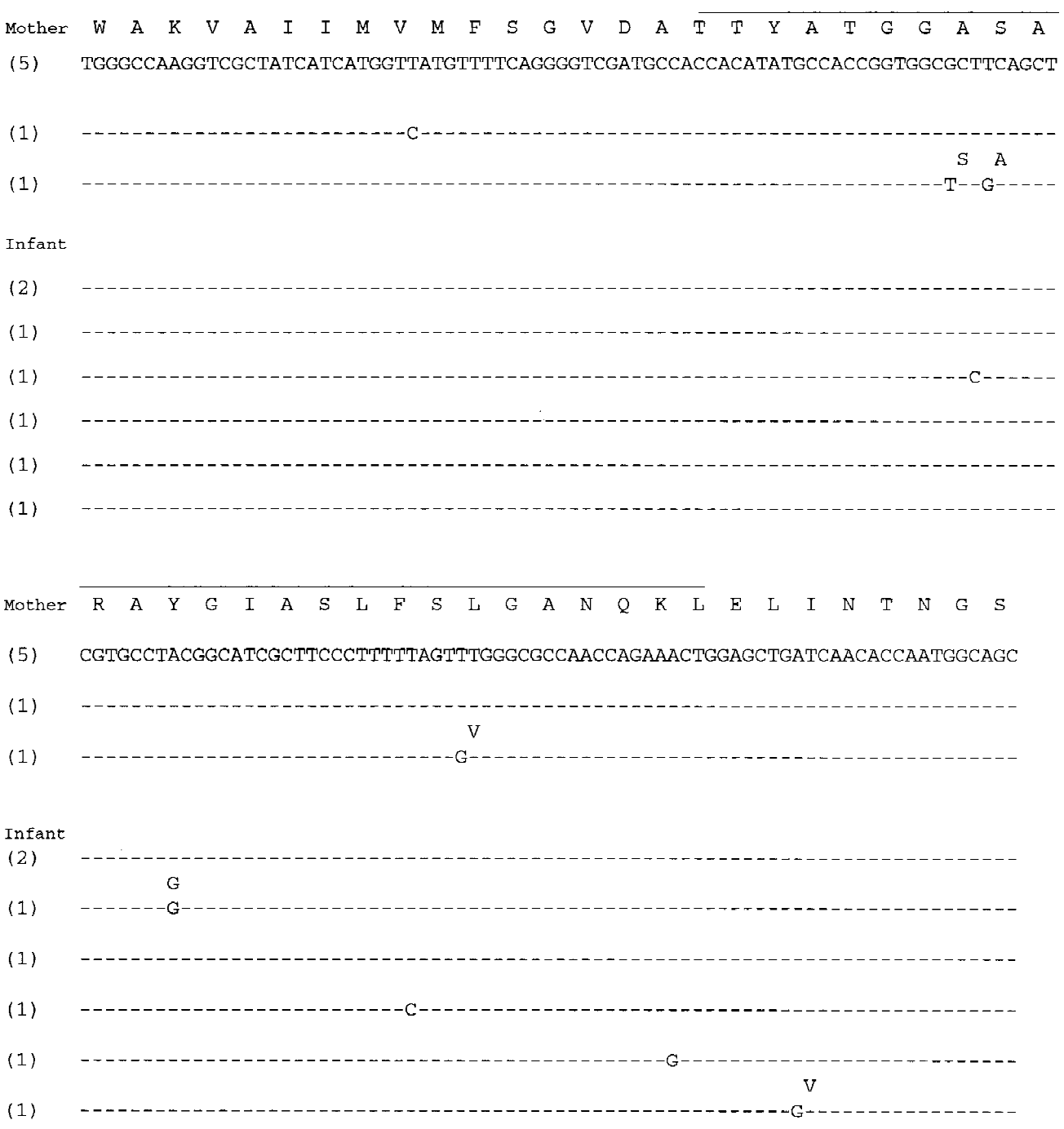

Fig. 3. Sequences of the E1–E2 junction (amino acids 368–418) derived from a mother–infant pair infected with genotype 3. The amino acid sequence is shown in the upper line, the corresponding nucleotide sequence in the lower line. The continuous line indicates the HVR1 sequence. Dashes represent nucleotide identities. The number of clones with each sequence is shown in parentheses.

seronegative HCV infection in infants of HCV-infected mothers remains a matter of controversy [Thaler et al., 1991; Manzini et al., 1995]. In the present study, children with vertical coinfection with HCV and HIV displayed weak anti-HCV reactivity with early loss of antibodies or delayed seroconversion. In two cases, anti-HCV was persistently negative until 11 and 26 months, respectively, despite repeated detection of HCV RNA. This shows that seronegative HCV infection may occur in babies coinfected with HIV. This observation may not be the rule, since previous reports showed the presence of anti-HCV in children with double infection [Novati et al., 1992; Zanetti et al., 1995; Manzini et al., 1995]. However, the results indicate that screening for HCV infection in HIV-infected children should include the determination of serum HCV RNA.

Hepatitis C is rare in childhood, and the natural history of the disease is poorly documented. In this study, five out of six vertically infected children did not become icteric and showed only mild elevations of ALT activities. One infant, coinfected with HIV, died at the age of 11 months with signs of cholestatic hepatitis. In children with perinatal HIV infection hepatitis has been associated with a shorter survival [Tovo et al., 1992] and has been related to the presence of multiple viral infections [Nigro et al., 1993]. However, the role played by HCV in liver disease of HIV-infected children is unclear at present.

The hypervariable region 1 (HVR1) of the E2 gene is remarkably heterogeneous [Weiner et al., 1992] and can be considered as specific to the individual in which it is found. The detection of identical HVR1 sequences in distinct patients can therefore indicate a common source of infection or person-to-person HCV transmission. We isolated identical HVR1 sequences from a mother–infant pair infected with HCV genotype 3 and without HIV coinfection, thus providing, consistent with previous studies [Aizaki et al., 1996; Weiner et al., 1993], molecular evidence of HCV transmission from an anti-HIV–negative mother. The HVR1 sequences detected in the baby's serum were heterogeneous and partly distinct from those detected in the mother. This observation is in contrast with data obtained in mother-to-infant HIV transmission, where sequences of the variable V3 loop are homogeneous in children upon primary infection [Wolinsky et al., 1992]. The distinct HVR1 variants isolated from the baby's blood could represent minor populations present but undetectable in the mother. Alternatively, since HVR1 sequence rapidly changes during the infection under the host immune pressure [Weiner et al., 1992] and since the sample tested was collected at 3 months, the HVR1 mutations might be the result of sequence evolution in the infected baby.

Mother-to-infant HCV transmission might occur in utero, during labour and delivery, or after birth through breast-feeding. By analogy to other viral infections, the detection of HCV genome shortly after birth could imply intrauterine transmission, whereas failure to detect HCV RNA could indicate perinatal infection. All newborns tested in this study were negative for HCV RNA, which was first detected at 2 months or later. In addition, the HVR1 master sequence detected in a 3-month-old infant was identical with the one identified in the mother at delivery. These results support the hypothesis of perinatal infection and might provide a rationale for preventive strategies reducing intrapartum or postpartum transmission. However, caesarean section was not apparently associated with lower rate of infection, and increased frequency of transmission was observed by HIV-infected mothers that avoided breast-feeding. These factors do not support a relationship between the type of delivery or feeding and the occurrence of vertical HCV infection, but larger prospective studies are required.

The results of this study confirm that mother-to-infant HCV transmission, although infrequent, may occur independent of the HIV status of the mother. By 6 months of age, the disappearance of anti-HCV or the loss of RIBA reactivities compared to the maternal profile can be useful to rule out vertical HCV infection in HIV-negative children. The determination of serum HCV RNA is useful for screening for vertical infection when anti-HCV persists after 6 months and in babies coinfected with HIV, who may show early and persistent loss of anti-HCV.

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